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NASA CR-184

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THE EFFECT OF BEDREST ON VARIOUS PARAMETERS OF PHYSIOLOGICAL FUNCTION

PART XIV. EFFECT OF BEDREST ON PLASMA LEVELS AND URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS

by D. Cardus, C. Vallbona, F. B. Vogt, W. A. Spencer, H. S. Lipscomb, and K. B. Eik-Nes

Prepared under Contract No. NAS 9-1461 *by*
TEXAS INSTITUTE FOR REHABILITATION AND RESEARCH
Houston, Texas
for

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION • WASHINGTON, D. C. • APRIL 1965

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ABSTRACT

Plasma levels of 17-hydroxycorticosteroids at 0800, 1200, 1600, 2000 and 2400 hours were determined on six healthy subjects who were submitted to bedrest for 3 days. The determinations were made with a modification of the Peterson method and the Porter-Silber technique. During bedrest the peak level at 0800 seemed a little lower than normal, but the circadian rhythm of 17-hydroxycorticosteroids was not modified. During bedrest and isometric exercises, the rhythm and the levels of 17-hydroxycorticosteroids were normal. Relatively short periods of physical inactivity seem to have no effect on the circadian rhythm of 17-hydroxycorticosteroids.

FORWORD

This study is a part of a NASA investigation of the effect of bedrest on various parameters of physiological function. It was sponsored by NASA Manned Spacecraft Center under Contract NAS-9-1461, with Dr. Lawrence F. Dietlein, Chief, Space Medicine Branch serving as Technical Monitor.

This study was conducted in the Immobilization Study Unit of the Texas Institute for Rehabilitation and Research, The Texas Medical Center. The following authors are affiliated with Baylor University College of Medicine as follows: Dr. Cardus, Departments of Rehabilitation and Physiology; Dr. Vallbona, Departments of Rehabilitation, Physiology, and Pediatrics; Dr. Vogt, Department of Rehabilitation; Dr. Spencer, Department of Rehabilitation; and Dr. Lipscomb, Department of Physiology. Dr. Eik-Nes is affiliated with the Department of Biological Chemistry, University of Utah, College of Medicine, Salt Lake City, Utah. It is through Dr. Eik-Nes' contribution that this study was also supported in part by NASA Manned Spacecraft Center Contract NAS-9-1294.

The authors wish to make acknowledgement of the contributions of Miss Rita Rapp of the Space Medicine Branch of the Manned Spacecraft Center of NASA, Mrs. Judy Brock and Mrs. Maribeth Cran in their technical assistance in collecting and handling the blood and urine samples; of Mrs. Angela Fraga in the statistical analysis; and Mrs. Patsy Dominy and Mrs. Jacqueline Clark in the preparation and typing of the manuscript.

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SUMMARY

This study was carried out to assess the possible effect of bedrest on plasma levels of 17-hydroxycorticosteroids.

Plasma levels of 17-hydroxycorticosteroids at 0800, 1200, 1600, 2000 and 2400 hours were determined on six healthy subjects who were submitted to bedrest for 3 days. Free and conjugated 17-hydroxycorticosteroids were determined in aliquots of 24 - hour samples of urine. The study was carried out in two periods. In the first, the subjects were submitted to bedrest. In the second, they followed a program of isometric exercises while in bedrest. The determinations were made with a modification of the Peterson method and the Porter-Silber technique for colorimetric reaction.

During bedrest, the peak level at 0800 seemed a little lower than normal, but the circadian rhythm of 17-hydroxycorticosteroids was not modified. During bedrest and isometric exercises, the rhythm and absolute levels of 17-hydroxycorticosteroids were normal. Relatively short periods of physical inactivity seem to have no effect on the circadian rhythm of 17-hydroxycorticosteroids.

INTRODUCTION

Bliss et al. (1) and Migeon et al. (2) have described a circadian rhythm of the 17-hydroxycorticosteroid plasma levels. Laidlaw et al. (3) described a similar circadian rhythm in the urinary excretion of 17-hydroxycorticosteroids. This rhythm of the adrenocortical function appears to be reflected in the pinnal mitosis and hepatic metabolism of phospholipids in the mouse, the number of eosinophils in the blood of human beings and the mouse (4), and in the electroencephalographic "output" of man. (5) Although this rhythm may change, Halberg (6) has stated that the timing, not the periodicity as such, of the circadian adrenal cycle is a function of the subject's routine of living.

The purpose of this investigation was to find out whether bedrest immobilization

had an effect on the circadian rhythm of plasma levels of 17-hydroxycorticosteroids of healthy subjects and to detect the possible effect of isometric exercise on this rhythm.

MATERIALS AND METHOD

This study was carried out in two periods. In the first, six subjects were studied from May 1 to May 11, 1963, and were submitted to bedrest immobilization on May 7, 8, and 9. In the second, the same subjects were studied from May 15, to May 25, 1963, and were submitted to bedrest immobilization with an added isometric exercise schedule on May 21, 22, and 23. During the period of bedrest the subjects were allowed to turn over in bed, to use one pillow under their head and to read and feed themselves. They were not allowed to sit in bed, raise their arms and legs above head level, or get up for any reason. The program of isometric exercises will be described in a separate report. Tilt tests were conducted on May 6, 9, 20, and 23. Some anthropometric characteristics and the usual occupation of these subjects are given in Table I.

Plasma determination of 17-hydroxycorticosteroids were made on samples drawn at 0800, 1200, 1600, 2000, and 2400 hours of the day using a modification of the Porter-Silber technique. Urinary 17-hydroxycorticosteroids were determined with a modification of the Peterson method using the Porter-Silber technique for the colorimetric reaction. Urine specimens were collected for 24 - hour periods. Urine volumes were recorded and aliquoted for various other determinations. Urine samples for steroid analysis were stored in toluene and refrigerated until the determinations were performed. Heparinized blood samples were collected by repetitive venous punctures. These samples were centrifuged immediately after collection, and the plasma was frozen.

RESULTS

Plasma levels of 17-hydroxycorticosteroids obtained on the six healthy subjects are summarized in Tables 2, 3, and 4. Table 2 contains the values observed while the subjects were ambulatory. This table shows the values for each individual sampled at consecutive 4-hour intervals (0800, 1200, 1600, 2000, and 2400 hours of the day) throughout the days that observations were made. Also, the mean and deviation values per hour and day of observation are given.

Table 3 shows the individual values at each hour of collection for May 8 (bedrest alone) and May 22 (bedrest with isometric exercise). Table 4 shows the average values of 17-hydroxycorticosteroids of the samples drawn at 0800, 1200, 1600, 2000, and 2400 hours of the day for all the subjects when they were ambulatory; mean values are given with the standard error and the standard deviation of the mean. The observations made during the days the subjects were submitted to the tilt test are not included in Table 4 because the procedure, or the emotional reaction to it, seemed to modify the pattern of 17-hydroxycorticosteroid levels in plasma. Table 4 also contains the average values observed when the subjects were bedridden. Figure 1 shows the average circadian rhythm of plasma 17-hydroxycorticosteroids observed when the subjects were bedridden and when ambulatory.

TABLE I

SUBJECTS PARTICIPATING IN THE EXPERIMENT

Subject		Age	Height	Weight	Body Surface	Usual
TIRR No.	Name	(years)	(centimeters)	(kilograms)	Area (m^2)	Occupation
70-0-06	C.B.B.	39	177	75.0	1.92	Process Operator*
70-0-09	T.H.L.	37	180	78.1	1.98	Process Operator*
70-0-07	R.N.M.	21	177	72.7	1.90	Clerk
70-0-08	W.R.S.	21	190	75.0	2.02	Student
70-0-01	R.K.W.	27	183	81.8	2.04	Newswriter
70-0-10	R.G.W.	40	175	76.8	1.92	Process Operator*

* On strike

TABLE 2

PLASMA 17 - OH - CS (γ / 100 ml.)

OBSERVED WHILE SUBJECTS WERE AMBULATORY

Subject	Time	5/3	5/10	5/17	5/24	$\bar{X} \pm$ S. D.
C.B.B.	0800	23.2	22.2	11.6	13.7	17.7 ± 5.88
T.H.L.		12.7	12.5	7.2	18.7	12.8 ± 4.70
R.N.M.		17.3	12.9	9.3	22.6	15.5 ± 5.74
W.R.S.		13.8	8.5	10.2	19.4	13.0 ± 4.82
R.K.W.		17.1	11.6	7.9	17.9	13.6 ± 4.73
R.G.W.		25.6	10.3	11.9	12.9	15.2 ± 7.04
$\bar{X} \pm$ S. D.		18.3±5.13	13.0±4.78	9.7±1.92	17.5±3.66	
C.B.B.	1200	3.1	3.7	4.1	5.5	4.8 ± 1.14
T.H.L.		8.5	11.3	1.8	16.8	9.6 ± 6.23
R.N.M.		7.0	10.3	6.2	8.1	7.9 ± 1.79
W.R.S.		3.3	4.0	1.9	6.4	3.9 ± 1.90
R.K.W.		4.7	11.0	5.7	17.9	9.8 ± 6.05
R.G.W.		6.6	4.8	5.5	9.0	6.5 ± 1.84
$\bar{X} \pm$ S. D.		6.0±1.82	7.5±3.70	4.2±1.95	10.6±5.37	
C.B.B.	1600	9.5	5.8	4.7	4.4	6.1± 2.35
T.H.L.		7.2	7.8	3.2	10.8	7.3 ± 3.12
R.N.M.		9.3	8.1	10.1	10.0	9.4 ± 0.89
W.R.S.		7.8	4.9	3.1	10.1	6.5 ± 3.10
R.K.W.		7.3	9.0	8.6	8.3	8.3 ± 0.71
R.G.W.		8.8	9.2	6.9	11.7	9.2 ± 1.98
$\bar{X} \pm$ S. D.		8.3±1.01	7.5±1.75	6.1±2.91	9.2±2.61	
C.B.B.	2000	7.8	4.7	3.0	7.2	5.7 ± 2.24
T.H.L.		2.9	3.6	3.8	14.9	6.3± 5.74
R.N.M.		14.3	4.3	1.0	7.7	6.8 ± 5.68
W.R.S.		3.3	7.0	5.9	5.7	5.5 ± 1.55
R.K.W.		7.3	5.5	5.0	7.3	6.3 ± 1.18
R.G.W.		7.3	4.2	3.5	7.0	5.5 ± 1.92
$\bar{X} \pm$ S. D.		7.2±4.46	4.9±1.21	3.7±1.69	8.3±3.30	
C.B.B.	2400	3.2	1.0	2.0	1.7	2.0 ± 0.89
R.H.L.		5.0	2.6	3.1	5.5	4.1 ± 1.41
R.N.M.		6.1	10.0	3.4	3.7	5.8 ± 3.05
W.R.S.		- -	0.4	0.9	1.6	1.0 ± 0.55
R.K.W.		1.6	2.3	2.2	2.9	2.3 ± 0.55
R.G.W.		2.6	3.8	4.3	5.6	4.1 ± 1.27
$\bar{X} \pm$ S. D.		3.7±1.82	3.4±3.47	2.7±1.20	3.5±1.77	

TABLE 3

PLASMA 17 - OH - CS (γ/ 100 ml.)

OBSERVED WHILE SUBJECTS WERE BEDRIDDEN

Subject	Time	5/8 Bedrest	5/22 Bedrest and Isometric Exercise	$\bar{X} \pm$ S. D.
CBB	0800	13.4	21.4	17.4 ± 5.66
THL		10.9	12.9	11.9 ± 1.41
RNM		13.1	13.0	13.1 ± 0.00
WRS		7.0	13.2	10.1 ± 4.38
RKW		11.6	9.5	10.6 ± 1.49
RGW		10.8	15.3	13.1 ± 3.18
$\bar{X} \pm$ S. D.		11.10 ± 2.30	14.60 ± 3.98	
CBB	1200	--	6.9	
THL		--	7.4	
RNM		--	14.8	
WRS		--	4.0	
RKW		--	6.3	
RGW		--	8.4	
$\bar{X} \pm$ S. D.		--	7.97 ± 3.66	
CBB	1600	7.7	14.1	10.9 ± 4.53
THL		9.7	8.4	9.1 ± 0.92
RNM		7.1	8.5	7.8 ± 0.99
WRS		6.0	6.5	6.3 ± 0.35
RKW		5.0	7.7	6.4 ± 1.91
RGW		8.0	8.2	8.1 ± 0.14
$\bar{X} \pm$ S. D.		7.25 ± 1.64	8.90 ± 2.65	
CBB	2000	6.1	4.8	5.5 ± 0.92
THL		6.3	5.5	5.9 ± 0.57
RNM		9.4	6.8	8.1 ± 1.84
WRS		6.5	5.2	5.9 ± 0.92
RKW		6.2	6.0	6.1 ± 0.14
RGW		4.5	6.1	5.3 ± 1.13
$\bar{X} \pm$ S. D.		6.50 ± 1.59	5.73 ± 0.72	
CBB	2400	1.9	1.0	1.5 ± 00.63
THL		2.3	1.0	1.7 ± 00.92
RNM		15.9	1.5	9.2 ± 10.18
WRS		3.7	3.0	3.4 ± 00.49
RKW		3.4	3.2	3.3 ± 00.14
RGW		3.8	2.8	3.3 ± 00.71
$\bar{X} \pm$ S. D.		5.17 ± 5.32	2.08 ± 1.03	

TABLE 4

AVERAGE PLASMA 17-HYDROXYCORTICOSTEROIDS (γ / 100 ml.)

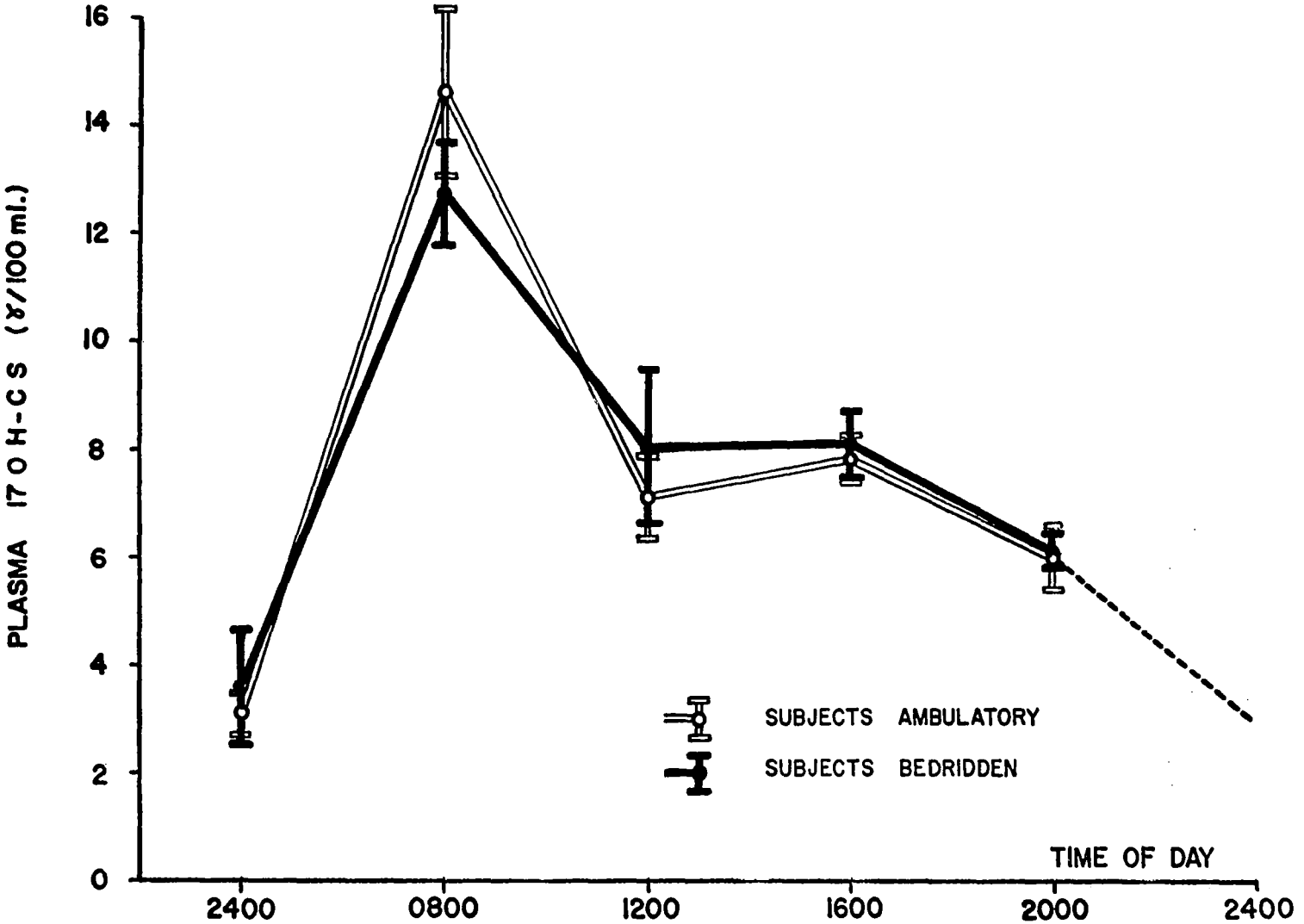
OBTAINED ON THE SIX SUBJECTS OF THIS STUDY

AT 0800, 1200, 1600, 2000, AND 2400 HOURS OF THE DAY

Time	Subjects Ambulatory		Subjects Bedridden	
	n	Mean ± S. E. S. D.	n	Mean ± S. E. S. D.
0800	24	14.6 ± 1.06 5.21	12	12.7 ± 1.01 3.49
1200	24	7.1 ± 0.83 4.07	6	8.0 ± 1.49 3.66
1600	24	7.8 ± 0.48 2.36	12	8.1 ± 0.66 2.27
2000	24	6.0 ± 0.66 3.23	12	6.1 ± 1.15 1.24
2400	23	3.1 ± 0.44 2.13	12	3.6 ± 1.15 3.99

FIGURE 1

CIRCADIAN RHYTHM OF PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS



EACH POINT AND BAR REPRESENTS THE MEAN AND ITS STANDARD ERROR

An analysis of variance of the 17-hydroxycorticosteroid values obtained at 0800, 1200, 1600, 2000, and 2400 hours of the day, and for each day including the days where tilt tests were performed, showed that the differences in the means of the samples obtained at 0800 hours were significant ($p < 0.01$), those of samples obtained at 1200 hours were perhaps significant ($0.01 < p < 0.05$), and those of the samples obtained at 1600, 2000, and 2400 hours were not significant ($p > 0.05$).

The determinations of 17-hydroxycorticosteroids in urine are reported in Tables 5 and 6. Table 5 shows the daily 24 - hour excretion of free, conjugated, and total 17-hydroxycorticosteroids while the subjects were ambulatory. Table 6 shows the daily 24 - hour excretion of free, conjugated, and total 17-hydroxycorticosteroids for May 7 (bedrest alone) and May 22 (bedrest with isometric exercise).

DISCUSSION

The average plasma levels of 17-hydroxycorticosteroids found in this investigation agree well with those reported by Doe et al. (7) on 13 healthy subjects. His values were the following: at 0600, 11.0 gammas per 100 milliliters; at 0900, 12.7 gammas per 100 milliliters; at 1500, 9.4 gammas per 100 milliliters; at 2100, 5.5 gammas per 100 milliliters. This pattern that consists of a peak around 0800 hours and a progressive decrease of 17-hydroxycorticosteroid plasma levels thereafter to midnight is also apparent in a diagram published by Frank (5) where he plots 17-hydroxycorticosteroids and the electroencephalogram "output." Perkoff et al. (8) have observed a secondary peak around 1600 hours. This secondary peak was also observed in the majority of the subjects included in this study, but it is much less apparent in the average values because of differences in phasing among individuals. Doe et al. (7) also reported that the daily average value for all his subjects was 10.1 gammas per 100 milliliters with a standard deviation of 4.3. The integrated values of the determinations made in this study varied from one subject to another (from 6.0 to 9.1 gammas per 100 milliliters).

Migeon et al. (2) reported that partial reversal of day-night activities did not modify the circadian rhythm of plasma 17-hydroxycorticosteroids in night workers. He also reported that studies carried out on blind persons showed that the stimulation from the sunlight was not an important, initiating factor of the rhythm. Benjamin and Crabbé (9) found lower concentrations of plasma 17-hydroxycorticosteroids in the 0800 hours blood sample of subjects who had been deprived of sleep for one night. Perkoff et al. (8) found that there is also a reversal of the circadian rhythm of the 17-hydroxycorticosteroids in plasma if there is a complete reversal of the sleep and activity pattern. Perkoff et al. (8) concluded that the relative importance of sleep versus activity was uncertain, although in various diseases resulting in prolonged inactivity, the direction and degree of the diurnal rhythm of the plasma 17-hydroxycorticosteroid levels were found to be normal.

TABLE 5

URINE FREE, CONJUGATED AND TOTAL 17-HYDROXYCORTICOSTEROIDS (Mg. in 24 Hours)

OBSERVED WHILE THE SUBJECTS WERE AMBULATORY

Subject	5/4			5/17			5/24			Individual Average		
	Free	Conj.	Total	Free	Conj.	Total	Free	Conj.	Total	Free	Conj.	Total
C.B.B.	-	-	-	0.097	7.53	7.627	0.052	3.22	3.272	0.075	5.37	5.450
T.H.L.	0.158	5.43	5.588	0.041	3.75	3.791	0.100	4.96	5.060	0.100	4.71	4.813
R.N.M.	0.133	5.44	5.573	0.066	3.85	3.916	0.085	4.61	4.695	0.095	4.63	4.728
W.R.S	-	-	-	0.045	2.45	2.495	0.083	3.02	3.103	0.064	2.73	2.799
R.K.W.	0.133	7.76	7.893	-	-	-	0.219	8.18	8.399	0.176	7.97	8.146
R.G.W.	0.088	2.72	2.808	0.074	3.63	3.704	0.062	2.77	2.832	0.075	3.04	3.115
Day Average	0.128	5.34	5.47	0.065	4.24	4.31	0.100	4.46	4.56			

TABLE 6

URINE FREE, CONJUGATED AND TOTAL 17-HYDROXYCORTICOSTEROIDS (Mg. in 24 Hours)

OBSERVED WHILE THE SUBJECTS WERE BEDRIDDEN

Subject	Bedrest 5/7			Bedrest with isometric exercise 5/22			Individual Average		
	Free	Conj.	Total	Free	Conj.	Total	Free	Conj.	Total
C.B.B.	0.156	4.21	4.366	0.136	3.53	3.666	0.146	3.87	4.016
T.H.L.	0.187	4.54	4.727	0.109	4.29	4.399	0.148	4.42	4.563
R.N.M.	0.110	3.64	3.750	0.160	5.28	5.440	0.135	4.46	4.595
W.R.S.	-	-	-	0.075	2.97	3.045	0.075	2.97	3.045
R.K.W.	0.230	8.32	8.550	0.151	8.22	8.371	0.191	8.27	8.461
R.G.W.	0.175	2.99	3.165	0.094	3.26	3.354	0.135	3.13	3.260
Day Average	0.172	4.74	4.912	0.121	4.59	4.713			

More recently, Halberg (6) has reported that in men under conditions of continued mental and moderate physical activity for two days, the length of the adrenal cycle shortened somewhat, its amplitude increased, and there was a rise in the over-all level around which cycling continued.

In comparing the average values obtained while the subjects were ambulatory with those obtained while the subjects were in bedrest (excluding the observations of the days that the subjects were tilted), this study shows that the circadian rhythm of 17-hydroxycorticosteroids in plasma was not altered by relatively short periods of bedrest immobilization. In spite of including the values obtained on the days that isometric exercise was added to bedrest, it seems that the peak values at 0800 hours were a little lower during bedrest than when the subjects were ambulatory, but the scarce number of observations made during bedrest does not permit one to draw any firm conclusion in this respect. The values observed on May 3 and on May 24, 1963, are considerably higher than the values observed during bedrest. This may be attributed to an emotional factor since these days were the first and the last days of the study. An alternative interpretation is that there is a superimposed cycle of longer periodicity in addition to the daily cycle. This might explain the fact that under comparable conditions the average values on May 24 were higher than on May 10. The second peak was more apparent when the subjects were ambulatory than when they were in bedrest. It was also lower the day following the two periods of bedrest. The daily pattern and average values observed during bedrest with isometric exercises did not seem to be different from those observed while the subjects were ambulatory.

There are four possibilities of combining normal sleep pattern and altered sleep pattern with activity and inactivity. Three of them have been tested. Normal sleep pattern with normal physical activity gives the normal pattern (Bliss' and Migeon's studies), normal sleep pattern with inactivity (this study) also gives a normal pattern, and reversal of sleep pattern with normal physical activity (Perkoff's study) gives a reversal of the circadian rhythm of 17-hydroxycorticosteroids in plasma. It would appear, therefore, that the alteration in the time pattern of sleep rather than the pattern of physical activity is an important factor in modifying the circadian rhythm of plasma levels of 17-hydroxycorticosteroids.

The measurement of free, as opposed to conjugated, 17-hydroxycorticosteroids gives no indication that one determination is preferable to the other to study circadian changes in urine.

CONCLUSIONS

1. Bedrest with the normal time pattern of sleep did not modify the circadian rhythm of plasma levels of 17-hydroxycorticosteroids.

2. Although the circadian rhythm was preserved, the peak level of 17-hydroxycorticosteroids in plasma at 0800 hours seemed to be smaller when the subjects were submitted to bedrest.

3. When the subjects were submitted to bedrest with isometric exercises, the pattern and the absolute levels of 17-hydroxycorticosteroids in plasma were not different from those observed when the subjects were ambulatory.

4. A review of studies conducted by other investigators on the effect of different factors on the circadian rhythm of plasma levels of 17-hydroxycorticosteroids and the results obtained in this investigation indicate that a relatively short period of physical inactivity is not a major modifying factor of this rhythm.

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